P. ENT COOPERATION TREA.

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room 524
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 26 October 2000 (26.10.00)	
International application No. PCT/US00/07995	**Applicant's or agent's file reference** 10365/07302
International filing date (day/month/year) 23 March 2000 (23.03.00)	**Priority date** (day/month/year) 23 March 1999 (23.03.99)
Applicant SIDHU, Rajinder, S. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 September 2000 (18.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Christelle Croci
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
10365/07302		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 07995	23/03/2000	23/03/1999
Applicant		
CYTOCLONAL PHARMACEUTICS, INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 00/07995

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C07K14/37 C12Q1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, BIOTECHNOLOGY ABS, SCISEARCH, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEERAKOON N D ET AL: "Isolation and characterization of the single beta-tubulin gene in Phytophthora cinnamomi." MYCOLOGIA, vol. 90, no. 1, January 1998 (1998-01), pages 85-95, XP000929307 ISSN: 0027-5514 cited in the application	55-67, 70-72, 78,79
Y	the whole document	1-9, 11-20, 24-26, 28-36, 38-49, 51-53, 74-77, 80-84
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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2000	08/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LONG DAVID M ET AL: "In vivo addition of telomeric repeats to foreign DNA generates extrachromosomal DNAs in the taxol-producing fungus Pestalotiopsis microspora." FUNGAL GENETICS AND BIOLOGY, vol. 24, no. 3, August 1998 (1998-08), pages 335-344, XP000929414 , ISSN: 1087-1845 the whole document	1-9, 11-20, 24-26, 74,75, 80-82,97
Y	YOUNG D H ET AL: "Antifungal properties of taxol and various analogues." EXPERIENTIA (BASEL), vol. 48, no. 9, 1992, pages 882-885, XP000929422 ISSN: 0014-4754 cited in the application the whole document	28-36, 38-49, 51-53, 76,77, 83,84,97
Y	RAO SRINIVASA ET AL: "Characterization of the Taxol Binding Site on the Microtubule: 2-(m-azidobenzoyl)taxol photolabels a peptide (amino acids 217-231) of beta-tubulin." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 35, 1995, pages 20235-20238, XP002143078 ISSN: 0021-9258 cited in the application the whole document	1-9, 11-20, 24-26
A	NOGALES EVA ET AL: "Structure of the alphabeta tubulin dimer by electron crystallography." NATURE (LONDON), vol. 391, no. 6663, 8 January 1998 (1998-01-08), pages 199-203, XP002143079 ISSN: 0028-0836 the whole document	1-7, 11-20, 24-26
A	" Acremonium chrysogenum wild-type beta-tubulin." GENESEQ DATABASE ; ACESSION NUMBER R40226 ; JP5192157, XP002143080 the whole document	1-7, 11-20, 24-26

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MU J -H ET AL: "Analysis of beta-tubulin cDNAs from taxol-resistant Pestalotiopsis microspora and taxol-sensitive Pythium ultimum and comparison of the taxol-binding properties of their products." MOLECULAR AND GENERAL GENETICS, vol. 262, no. 4-5, December 1999 (1999-12), pages 857-868, XP002143081 ISSN: 0026-8925 the whole document	1-54

09/937052

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PATENT COOPERATION TREATY

PCT

REC'D 03 JUL 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
10365/07302		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/07995	23/03/2000	23/03/1999
International Patent Classification (IPC) or national classification and IPC C12N15/31		
Applicant CYTOCLONAL PHARMACEUTICS, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 13 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/09/2000	29.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bilang, J Telephone No. +49 89 2399 8707

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/07995

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-38 as originally filed

Claims, No.:

1-82 with telefax of 21/05/2001

Drawings, sheets:

1/10-10/10 as originally filed

Sequence listing part of the description, pages:

1-32, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
see separate sheet

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 2-4,6,8,11-13,15-17,19,21,24-27,29-31,33,35,38-40,42-44,46,48,5

	No:	Claims	1-54,62,63,71-77,80-98
			1,5,7,9,10,14,18,20,22,23,28,32,34,36,37,41,45,47,49,50,55-61,64-70,78,79
Inventive step (IS)	Yes:	Claims	12,13,25-27,39,40,52-54,63,73,80-86,89,90,92,98
	No:	Claims	1-11,14-24,28-38,41-51,55-62,64-72,74-79,87,88,91,93-97
Industrial applicability (IA)	Yes:	Claims	1-98
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Additional remarks Item I

1. The priority appears to be validly claimed.

2. This report is based on the claims as originally filed because the amendments of the claims as filed on 21.05.2001 are not based on the application as originally filed.

2.1 New claim 2 covers a DNA segment consisting of a fragment of SEQ ID NO: 1 having at least eighteen contiguous nucleotides. No basis could be found in the application as originally filed for such a segment. Such a fragment is also not directly and unambiguously derivable from Figure 8.

The same argument is valid for new claims 44 and 55.

2.2 New claim 3 is concerned with the DNA segment of claim 2 wherein the segment comprises the sequence from nucleotide 75 to 167 of SEQ ID NO: 1, wherein one or more nucleotide substitutions occur at specified positions (i.e. the substitutions are mandatory and the segment does not have the sequence of SEQ ID NO: 1). No basis could be found for such a substituted segment.

The same argument applies to new claims 5, 7, 13, 15, 17, 24, 26, 28, 38, 45, 47, 49, 56, 58, and 60.

In the application as originally filed the applicants compared the sequences of beta-tubulin derived from different organisms. However, it was not derivable from the application as originally filed that sequences having one or more substitutions at these positions were part of the invention. Moreover, specific substitutions were selected in a manner that was not derivable from the application as originally filed.

2.3 New claims 43, 54, and 78 define a beta-tubulin comprising at least three specific amino acids of the sequence shown in SEQ ID NO: 6.
No basis could be found for such a beta-tubulin in the application as originally filed. With this amendment the applicants attempt to exclude the beta-tubulin disclosed in D1 from the scope of said new claims. It should be noted, however,

that this disclaimer is not based on D1 since D1 does not disclose a beta-tubulin having only three of the eight amino acid substitutions.

2.4 Further amendments which go beyond the disclosure as originally filed may be present in the new set of claims. However, due to the number of unallowable amendments already identified this authority does not consider it to be expedient to perform a complete in-depth analysis of the claims.

3. Several claims appear to depend on claims on which they cannot depend. New claim 6, for example, depends on new claim 5 wherein the DNA segment has the sequence as shown in SEQ ID NO: 1 (nucleotide 708 to 764). The DNA segment of new claim 5, however, does not embrace the sequence shown in SEQ ID NO: 1 since at least one nucleotide substitution is mandatory.

4. The applicants provided a table comparing the subject-matter of the old claims with the subject-matter of the new claims. It should be noted, however, that the subject-matter of, for example, new claim 3 does not correspond to the subject-matter of old claim 7, because new claim 3 defines specific nucleotides which have to be substituted. Similarly, new claim 5 specifies nine nucleotide substitutions, whereas old claims 9 and 10 only specify three substitutions.

5. Due to the number of unallowable amendments, the unclarities introduced into the set of claims by the dependencies, and due to the unavailability of clear indications of the amendments and their basis, it is not possible to formulate a reasoned statement with respect to the novelty, inventive step or industrial applicability of the amended set of claims. Consequently, this report is based on the claims as originally filed.

Additional remarks Item IV

1. The international search has been drawn up in respect of the entire international application, but the IPEA finds that the application does not comply with the requirements of unity of invention (**Article 34(3)** and **Rules 13** and **68 PCT**).

2. An international application must relate to one invention only or to a group of

inventions so linked as to form a single general inventive concept.
Unity of invention is fulfilled only when there is a technical relationship among the inventions involving one or more of the same or corresponding special technical features, special technical features being such features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

The technical relationship among the present subject-matter of claims 1, 28, and 55 is that all claims relate to "DNA segment encoding an oomycete β-tubulin". However, this relation cannot be considered as the special technical feature as defined above because it is not novel. In fact, β-tubulin from an oomycete was known in the art (Weerakon et al., Mycologia, vol. 90, 1998, p. 85-95).

The contributions claimed in the present application which are allegedly made over the prior art are:

1. β-tubulin from Pestalotiopsis microspora and its use;
2. β-tubulin Phytophthora cinnamomi and its use;
3. β-tubulin Phytophthora cinnamomi and its use

These contributions are not so linked as to form one single inventive concept. Hence, the present claims fall apart into at least three groups of inventions.

Additional remarks Item V

1. The present application discloses the nucleotide and amino acid sequences of beta-tubulin from three different oomycetes, Pestalotiopsis microspora, Pythium ultimum, and Phytophthora cinnamomi. The tubulin of P. microspora is taxol-resistant, whereas the tubulin of the latter two oomycetes in taxol-sensitive. The application furthermore discloses that a particular amino acid (219) of beta-tubulin can serve as an indicator of tubulin-resistance or -sensitivity.

2. The following documents were taken into consideration:

   D1 (Weerakon et al., Mycologia, vol. 90, 1998, p. 85-95) discloses the isolation and characterization of the beta-tubulin gene of Phytophthora cinnamomi. It also

discloses that this beta-tubulin gene shares 98% sequence identity with a beta-tubulin gene from another Oomycete.

D2 (Young et al., Experientia, vol. 48, 1992, p. 882-885) discloses that the oomycete P. capsici is sensitive to taxol.

Contrary to P. capsici, P. microspora must be suspected to be resistant to taxol because it produces taxol itself (D3: Long et al., Fungal Genetics and Biology, vol. 24, 1998, p. 335-344; and US 5,861,302, cited in the application as the source of P. microspora).

D4 (Rao et al., The Journal of Biological Chemistry, vol. 270, 1995, p. 20235-20238) discloses a peptide of beta-tubulin to which taxol binds. These findings were confirmed in D5 (Nogales et al., Nature, vol. 391, issue of 08.01.1998, p. 199- 203).

3. None of the available prior art documents appears to disclose or suggest that Threonine 219 would be crucial for sensitivity or resistance to taxol. The studies disclosed in D4 and D5 identified a peptide containing residues 217-231 of beta-tubulin derived from bovine brain which binds to taxol. However, these documents do not appear to draw the attention to any particular residue within this peptide. Moreover, it could not necessarily be concluded that the binding site would be at the same positions in fungal beta-tubulin.

4. However, in view of the disclosures of D1, the IPEA considers that the subject-matter of claims 1, 5, 7, 9, 10, 14,18, 20, 22, 23, 28, 32, 34, 36, 37, 41, 45, 47, 49, 50, 55- 61, 64-70, 78, and 79 has been anticipated by the prior art and therefore contravene **Article 33(2)(3) PCT**.

4.1 Claim 55 covers a DNA segment encoding a beta-tubulin of P. cinnamomi or a portion thereof, consisting essentially of at least a portion of SEQ ID NO: 5

The DNA sequence of the beta-tubulin of P. cinnamomi was known from D1, which thus anticipate the subject-matter of claim 55.

The same argument is valid for claims 56, 58, 60, 64, 65, 67, 70, 78, and 79.

4.2 Claim 1 covers a DNA segment encoding a portion of a beta-tubulin.
One amino acid is considered to be a portion of a given protein. In its present formulation claim 5 thus embraces any DNA segment.
It should be noted that the source of a DNA segment is irrelevant if said DNA segment is identical to an already known segment.

The same objections apply to claims 5, 14, 18, 28, 32, 41, 45, 55, and 64.

4.3 According to claim 7 at least one nucleotide is substituted. This wording embraces any DNA encoding beta-tubulin.

The same objection applies to claims 9, 10, 20, 22, 23, 34, 36, 37, 47, 49, 50, 57, 59, 61, 66, 68, and 69.

5 The subject-matter of claims 1-11, 14-22, 24, 28-38, 41-49, 51, 57, 59, 61, 62, 66, 68, 69, 71, 72, 74-77, 87, 88, 91, and 93-97 is not considered to be based on an inventive activity, contrary to **Article 33(3) PCT**.

5.1 Once the tubulin of D1 is known it does not require to provide variants thereof having at least one nucleotide changed but no further technical features. The subject-matter of claims 57, 59, 61, 62, 66, 68, 69, 71 and 72 thus can be obtained in an obvious manner.

5.2 Claims 91 and 93 embrace a fungal cell transformed with the beta-tubulin disclosed in D1. It does not require inventive activity to transform an organism with a known nucleic acid.

5.3 It can be understood from D1 that the DNA encoding beta-tubulin is well conserved between oomycetes (98% identity between P. cinnamomi and Achlya klebsiana). The skilled person would know that the sequences disclosed in D1 can be used to isolate beta-tubulin genes from other oomycetes. The subject-matters of claims 1-11, 14-22, 24, 28-38, 41-49, 51, and 74-77, insofar as novel, thus are not considered to be based on an inventive activity (**Article 33(3) PCT**).

5.4 It was known in the art that taxol acts on beta-tubulin. The methods of claims 87 and 88 thus are trivial once taxol resistant beta-tubulin is known.

5.5 The methods of claims 94-97 can be performed without having knowledge of the sequence of taxol resistant or taxol sensitive beta-tubulin. It appears that these methods are trivial to the skilled person who was aware of the mode of action of taxol and of the presence of sensitive and resistant microorganisms.

Additional remarks Item VIII

1. Claim 1 merely cites the technical problem which is to be solved ("DNA encoding tubulin"), without containing any true technical features of the claimed matter. Claim 1 should specify the matter for which protection is sought by its technical features, i.e. by its sequence.

   The same objection applies to claims 2-4, 12-17, 25-31, 39-44, 52-54, 62, 63, and 73.

2. The requirement of Article 5 PCT not only apply to an individual claim but also to the set of claims as a whole.
   Claim 7 appears to be a dependent claim, depending on claim 6 (which depends on claim 1). A closer look, however, reveals that the scope of claim 7 is much broader than the scope of claim 6, because the sequence which was defined in claim 6 may be changed again. Claim 7 thus is an independent claim.

   The same objection applies to most of the claims covering DNA or amino acid sequences.

3. According to claim 7 at least one nucleotide in the claimed sequence is substituted. It is not clear, however, with respect to what said nucleotide is substituted.

4. The present application does not appear to disclose antibodies that could distinguish between taxol-sensitive and -resistant tubulin. It is not clear whether such antibodies can be obtained, and thus whether the method according to

claims 80-86 can be put into practise.

5. Claim 88 covers a method comprising the step of providing mycelia as depicted in SEQ ID NO: 6. Said SEQ ID NO 6. does not depict a mycelia but an amino acid sequence.

6. Claim 89 covers a method of altering the taxol binding property of a recombinantly expressed beta-tubulin.
The present application does not provide any evidence that the taxol binding properties of e.g. bovine brain beta-tubulin would be modified if the amino acid corresponding to position 219 of SEQ ID NO: 2 would be modified.

7. The subject-matter of claim 93 appears to be identical to the subject-matter of claim 91.

We claim:

1. A purified DNA segment encoding a beta-tubulin of the fungal species *Pestalotiopsis microspora* or a portion thereof, wherein said beta-tubulin has an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:2 comprising at least one conservative modification thereof, and wherein said portion comprises one or more taxol binding sites selected from the group consisting of taxol binding site I and taxol binding site II.

2. The DNA segment of claim 1, wherein said DNA segment consists of a fragment of SEQ ID NO:1 having at least eighteen contiguous nucleotides.

3. The DNA segment of claim 2, wherein said portion comprises the nucleotide sequence from nucleotide 75 to nucleotide 167 of SEQ ID NO:1, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 129, nucleotide 130, nucleotide 131, nucleotide 138, nucleotide 139, nucleotide 140, nucleotide 141, nucleotide 142, nucleotide 143, nucleotide 147, nucleotide 148, and nucleotide 149.

4. The DNA segment of claim 3, wherein said portion comprises the nucleotide sequence from nucleotide 75 to nucleotide 167 of SEQ ID NO:1.

5. The DNA segment of claim 2, wherein said portion comprises the nucleotide sequence from nucleotide 708 to nucleotide 764 of SEQ ID NO:1, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 726, nucleotide 727, nucleotide 728, nucleotide 729, nucleotide 730, nucleotide 731, nucleotide 735, nucleotide 736 and nucleotide 737.

6. The DNA segment of claim 5, wherein said portion comprises the nucleotide sequence from nucleotide 708 to nucleotide 764 of SEQ ID NO:1.

7. The DNA segment of claim 2, wherein said portion consists of the nucleotide sequence from nucleotide 75 to nucleotide 1412 of SEQ ID NO:1, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 129, nucleotide 130, nucleotide 131, nucleotide 138, nucleotide 139, nucleotide 140, nucleotide 141, nucleotide

142, nucleotide 143, nucleotide 147, nucleotide 148, nucleotide 149, nucleotide 726, nucleotide 727, nucleotide 728, nucleotide 729, nucleotide 730, nucleotide 731, nucleotide 735, nucleotide 736 and nucleotide 737.

8. The DNA segment of claim 7, wherein said portion consists of the nucleotide sequence from nucleotide 75 to nucleotide 1412 of SEQ ID NO:1.

9. The DNA segment of claim 1, 3, 5 or 7, wherein said one or more nucleotide substitutions does not alter the taxol binding capacity of said portion.

10. The DNA segment of claim 1, 3, 5 or 7, wherein said one or more nucleotide substitutions alters the taxol binding capacity of said portion.

11. An amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Pestalotiopsis microspora*, wherein said beta-tubulin has an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:2 comprising at least one conservative modification thereof, and wherein said portion comprises one or more taxol binding sites selected from the group consisting of taxol binding site I and taxol binding site II.

12. The amino acid sequence of claim 11, wherein said portion consists of a fragment of SEQ ID NO:2 having at least six contiguous amino acids.

13. The amino acid sequence of claim 12, wherein said portion comprises the amino acid sequence from amino acid 1 to amino acid 31 of SEQ ID NO:2, wherein one or more amino acid substitutions occur in the group consisting of amino acid 19, amino acid 22, amino acid 23, and amino acid 25.

14. The amino acid sequence of claim 13, wherein said portion comprises the amino acid sequence from amino acid 1 to amino acid 31 of SEQ ID NO:2.

15. The amino acid sequence of claim 12, wherein said portion comprises the amino acid sequence from amino acid 212 to amino acid 230 of SEQ ID NO:2, wherein one or more amino acid substitutions occur in the group consisting of amino acid 218, amino acid 219 and amino acid 221.

16. The amino acid sequence of claim 15, wherein said portion comprises the amino acid sequence from amino acid 212 to amino acid 230 of SEQ ID NO:2.

17. The amino acid sequence of claim 12, wherein said portion consists of the amino acid sequence from amino acid 1 to amino acid 446 of SEQ ID NO:2, wherein one or more amino acid substitutions occur in the group consisting of amino acid 19, amino acid 22, amino acid 23, amino acid 25, amino acid 218, amino acid 219 and amino acid 221.

18. The amino acid sequence of claim 17, wherein said portion consists of the amino acid sequence from amino acid 1 to amino acid 446 of SEQ ID NO:2.

19. The amino acid sequence of claim 11, 13, 15 or 17, wherein said portion contains at least one amino acid substitution that alters the taxol binding property of said portion.

20. The amino acid sequence of claim 11, 13, 15 or 17, wherein said portion contains at least one amino acid substitution that does not alter the taxol binding property of said portion.

21. The amino acid sequence of claim 11, wherein said amino acid sequence is substituted with any amino acid which perturbs the three-dimensional structure of said amino acid sequence surrounding amino acid 219 as numbered in SEQ ID NO:2.

22. A purified DNA segment encoding a beta-tubulin of the fungal species *Pythium ultimum* or a portion thereof, wherein said beta-tubulin has an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:4 comprising at least one conservative modification thereof, and wherein said portion comprises one or more taxol binding sites selected from the group consisting of taxol binding site I and taxol binding site II.

23. The DNA segment of claim 22, wherein said DNA segment consists of a fragment of SEQ ID NO:3 having at least eighteen contiguous nucleotides.

24. The DNA segment of claim 23, wherein said portion comprises the nucleotide sequence from nucleotide 92 to nucleotide 184 of SEQ ID NO:3, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 146, nucleotide 147, nucleotide 148, nucleotide 155, nucleotide 156, nucleotide 157, nucleotide 158, nucleotide 159, nucleotide 160, nucleotide 164, nucleotide 165, and nucleotide 166.

25. The DNA segment of claim 24, wherein said portion comprises the nucleotide sequence from nucleotide 92 to nucleotide 184 of SEQ ID NO:3.

26. The DNA segment of claim 23, wherein the portion comprises the nucleotide sequence from nucleotide 725 to nucleotide 781 of SEQ ID NO:3, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 743, nucleotide 744, nucleotide 745, nucleotide 746, nucleotide 747, nucleotide 748, nucleotide 752, nucleotide 753 and nucleotide 754.

27. The DNA segment of claim 26, wherein the portion comprises the nucleotide sequence from nucleotide 725 to nucleotide 781 of SEQ ID NO:3.

28. The DNA segment of claim 23, wherein said portion consists of the nucleotide sequence from nucleotide 92 to nucleotide 1429 of SEQ ID NO:3, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 146, nucleotide 147, nucleotide 148, nucleotide 155, nucleotide 156, nucleotide 157, nucleotide 158, nucleotide 159, nucleotide 160, nucleotide 164, nucleotide 165, nucleotide 166, nucleotide 743, nucleotide 744, nucleotide 745, nucleotide 746, nucleotide 747, nucleotide 748, nucleotide 752, nucleotide 753 and nucleotide 754.

29. The DNA segment of claim 28, wherein said portion consists of the nucleotide sequence from nucleotide 92 to nucleotide 1429 of SEQ ID NO:3.

30. The DNA segment of claim 22, 24, 26 or 28, wherein said one or more nucleotide substitutions does not alter the taxol binding capacity of said portion.

31. The DNA segment of claim 22, 24, 26 or 28, wherein said one or more nucleotide substitutions alters the taxol binding capacity of said portion.

32. An amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Pythium ultimum*, wherein said portion has an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:4 comprising at least one conservative modification thereof, and wherein said portion comprises one or more taxol binding sites selected from the group consisting of taxol binding site I and taxol binding site II.

33. The amino acid sequence of claim 32, wherein said portion consists of a fragment of SEQ ID NO:4 having at least six contiguous amino acids.

34. The amino acid sequence of claim 33, wherein said portion comprises the amino acid sequence from amino acid 1 to amino acid 31 of SEQ ID NO:4, wherein one or more amino acid substitutions occur in the group consisting of amino acid 19, amino acid 22, amino acid 23, and amino acid 25.

35. The amino acid sequence of claim 34, wherein said portion comprises the amino acid sequence from amino acid 1 to amino acid 31 of SEQ ID NO:4.

36. The amino acid sequence of claim 33, wherein said portion comprises the amino sequence from amino acid 212 to amino acid 230 of SEQ ID NO:4, wherein one or more amino acid substitutions occur in the group consisting of amino acid 218, amino acid 219 and amino acid 221.

37. The amino acid sequence of claim 36, wherein said portion comprises the amino sequence from amino acid 212 to amino acid 230 of SEQ ID NO:4.

38. The amino acid sequence of claim 33, wherein said portion consists of the amino acid sequence from amino acid 1 to amino acid 446 of SEQ ID NO:4, wherein one or more amino acid substitutions occur in the group consisting of amino acid 19, amino acid 22, amino acid 23, amino acid 25, amino acid 218, amino acid 219 and amino acid 221.

39. The amino acid sequence of claim 38, wherein said portion consists of the amino acid sequence from amino acid 1 to amino acid 446 of SEQ ID NO:4.

40. The amino acid sequence of claim 32, 34, 36 or 38, wherein said portion contains at least one amino acid substitution that alters the taxol binding property of said portion.

41. The amino acid sequence of claim 32, 34, 36 or 38, wherein said portion contains at least one amino acid substitution that does not alter the taxol binding property of said portion.

42. The amino acid sequence of claim 32, wherein said amino acid sequence is substituted with any amino acid which perturbs the three-dimensional structure of said amino acid sequence surrounding amino acid 219 as numbered in SEQ ID NO:4.

43. A purified DNA segment encoding a beta-tubulin of the fungal species *Phytophthora cinnamomi* or a portion thereof, wherein said beta-tubulin has an amino acid sequence selected from the group consisting of SEQ ID NO:6 and SEQ ID NO:6 comprising at least one conservative modification thereof, and wherein said portion has at least three amino acids selected from the group consisting of amino acid 24, amino acid 219, amino acid 249, amino acid 251, amino acid 252, amino acid 253, amino acid 359 and amino acid 428, and wherein said portion comprises one or more taxol binding sites selected from the group consisting of taxol binding site I and taxol binding site II.

44. The DNA segment of claim 43, wherein said DNA segment consists of a fragment of SEQ ID NO:5 having at least eighteen contiguous nucleotides.

45. The DNA segment of claim 44, wherein said portion comprises the nucleotide sequence from nucleotide 11 to nucleotide 103 of SEQ ID NO:5, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 65, nucleotide 66, nucleotide 67, nucleotide 74, nucleotide 75, nucleotide 76, nucleotide 77, nucleotide 78, nucleotide 79, nucleotide 80, nucleotide 81, nucleotide 82, nucleotide 83, nucleotide 84, and nucleotide 85, provided that when said nucleotide substitutions result in a single amino acid change, nucleotide 80 to nucleotide 82 does not encode isoleucine.

46. The DNA segment of claim 45, wherein said portion comprises the nucleotide sequence from nucleotide 11 to nucleotide 103 of SEQ ID NO:5.

47. The DNA segment of claim 44, wherein said portion comprises the nucleotide sequence from nucleotide 644 to nucleotide 700 of SEQ ID NO:5, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 662, nucleotide 663, nucleotide 664, nucleotide 665, nucleotide 666, nucleotide 667, nucleotide 671, nucleotide 672 and nucleotide 673, provided that when said nucleotide substitutions result in a single amino acid change, nucleotide 665 to nucleotide 667 does not encode asparagine.

48. The DNA segment of claim 47, wherein said portion comprises the nucleotide sequence from nucleotide 644 to nucleotide 700 of SEQ ID NO:5.

49. The DNA segment of claim 44, wherein said fragment consists of the nucleotide sequence from nucleotide 11 to nucleotide 1342 of SEQ ID NO:5, wherein one or more nucleotide substitutions occur in the group consisting of nucleotide 65, nucleotide 66, nucleotide 67, nucleotide 74, nucleotide 75, nucleotide 76, nucleotide 77, nucleotide 78, nucleotide 79, nucleotide 80, nucleotide 81, nucleotide 82, nucleotide 83, nucleotide 84, nucleotide 85, nucleotide 662, nucleotide 663, nucleotide 664, nucleotide 665, nucleotide 666, nucleotide 667, nucleotide 671, nucleotide 672 and nucleotide 673, provided that when said nucleotide substitutions result in a single amino acid change, nucleotide 80 to nucleotide 82 does not encode isoleucine or nucleotide 665 to nucleotide 667 does not encode asparagine.

50. The DNA segment of claim 49, wherein said fragment consists of the nucleotide sequence from nucleotide 11 to nucleotide 1342 of SEQ ID NO:5.

51. The DNA segment of claim 43, 45, 47 or 49, wherein said one or more nucleotide substitutions does not alter the taxol binding capacity of said portion.

52. The DNA segment of claim 43, 45, 47 or 49, wherein said one or more nucleotide substitutions alters the taxol binding capacity of said portion.

53. The DNA segment of claim 1, 5, 22, 26, 43 or 47, wherein said portion is able to interact with alpha-tubulin.

54. An amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Phytophthora cinnamomi*, wherein said portion has an amino acid sequence selected from the group consisting of SEQ ID NO:6 and SEQ ID NO:6 comprising at least one conservative modification thereof, and wherein said portion has at least three amino acids selected from the group consisting of amino acid 24, amino acid 219, amino acid 249, amino acid 251, amino acid 252, amino acid 253, amino acid 359 and amino acid 428, and wherein said portion comprises one or more taxol binding sites selected from the group consisting of taxol binding site I and taxol binding site II.

55. The amino acid sequence of claim 54, wherein said portion consists of a fragment of SEQ ID NO:6 having at least six contiguous amino acids.

56. The amino acid sequence of claim 55, wherein said fragment comprises the amino acid sequence from amino acid 1 to amino acid 31 of SEQ ID NO:6, wherein one or more amino acid substitutions occur in the group consisting of amino acid 19, amino acid 22, amino acid 23, amino acid 24, and amino acid 25, provided that when only one amino acid is substituted, amino acid 24 is not isoleucine.

57. The amino acid sequence of claim 56, wherein said fragment comprises the amino acid sequence from amino acid 1 to amino acid 31 of SEQ ID NO:6.

58. The amino acid sequence of claim 55, wherein said fragment comprises the amino acid sequence from amino acid 212 to amino acid 230 of SEQ ID NO:6, wherein one or more amino acid substitutions occur in the group consisting of amino acid 218, amino acid 219 and amino acid 221, provided that when only one amino acid is substituted, amino acid 219 is not asparagine.

59. The amino acid sequence of claim 58, wherein said fragment comprises the amino acid sequence from amino acid 212 to amino acid 230 of SEQ ID NO:6.

60. The amino acid sequence of claim 55, wherein said fragment consists of the amino acid sequence from amino acid 1 to amino acid 446 of SEQ ID NO:6, wherein one or more amino acid substitutions occur in the group consisting of amino acid 19, amino acid 22, amino acid 23, amino acid 24, amino acid 25, amino acid 218, amino acid 219 and

amino acid 221, provided that when only one amino acid is substituted, amino acid 24 is not isoleucine or amino acid 219 is not asparagine.

61. The amino acid sequence of claim 60, wherein said fragment consists of the amino acid sequence from amino acid 1 to amino acid 446 of SEQ ID NO:6.

62. The amino acid sequence of claim 54, 56, 58 or 60, wherein said portion contains at least one amino acid substitution that alters the taxol binding property of said portion.

63. The amino acid sequence of claim 54, 56, 58 or 60, wherein said portion contains at least one amino acid substitution that does not alter the taxol binding property of said portion.

64. The amino acid sequence of claim 54, wherein said amino acid sequence is substituted with any amino acid which perturbs the three-dimensional structure of said amino acid sequence surrounding amino acid 219, provided that when only one amino acid is substituted at amino acid 219, the substituted amino acid is not asparagine.

65. The amino acid sequence of claim 11, 15, 32, 36, 54 or 58, wherein said portion is able to interact with alpha-tubulin.

66. A vector comprising a purified DNA segment of claim 1, 2, 22, 23, 43 or 44.

67. The vector of claim 66, wherein said portion encodes at least one taxol binding site.

68. A method of determining the taxol binding capacity of a beta-tubulin or beta-tubulin-like compound comprising

providing monoclonal antibodies raised against amino acid sequences comprising at least one taxol binding site of a beta-tubulin from taxol-resistant *Pestalotiopsis microspora* as depicted in SEQ ID NO:2, taxol-sensitive *Pythium ultimum* as depicted in SEQ ID NO:4, or taxol-sensitive *Phytophthora cinnamomi* as depicted in SEQ ID NO:6 to form a reagent;

contacting said beta-tubulin with said reagent; and

determining degree of binding between said monoclonal antibodies in said reagent and said beta-tubulin or beta-tubulin-like compound;

whereby bound monoclonal antibodies that specifically recognize a taxol-sensitive binding site of a beta-tubulin indicate taxol sensitivity and whereby bound monoclonal antibodies that specifically recognize a taxol-resistant binding site of a beta-tubulin indicate taxol resistance.

69. The method of claim 68, wherein said taxol-sensitive binding site is taxol binding site II with threonine at amino acid 219 as numbered in SEQ ID NO:2 and wherein said taxol-resistant binding site is taxol binding site II without threonine at amino acid 219 as numbered in SEQ ID NO:2.

70. The method of claim 68 or 69, wherein said monoclonal antibodies are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from said *Pestalotiopsis microspora* as depicted in SEQ ID NO:2.

71. The method of claim 68 or 69, wherein said monoclonal antibodies are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from said *Pythium ultimum* as depicted in SEQ ID NO:4.

72. The method of claim 68 or 69, wherein said monoclonal antibodies are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from said *Phytophthora cinnamomi* as depicted in SEQ ID NO:6.

73. The method of claims 68, 69, 70, 71 or 73, wherein said beta-tubulin or beta-tubulin-like protein is selected from the group consisting of recombinantly expressed protein, exogenously isolated protein, synthetic peptides, and cell cultures.

74. A method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising

providing taxol-binding beta-tubulins with amino acid sequences having threonine at amino acid 219 as numbered in SEQ ID NO:4, wherein said beta-tubulins are added to alpha-tubulin to form a reagent;

contacting said composition of matter with said reagent; and

determining the ability of said composition of matter to promote microtubulin assembly or prevent depolymerization of assembled microtubulins under depolymerizing conditions;

whereby the ability to promote microtubule assembly or prevent depolymerization indicates the possible presence of taxol or taxol-like compounds in said composition of matter.

75. A method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising

generating taxol-sensitive and taxol-resistant isogenic fungal cells from one of the species *Pestalotiopsis microspora*, *Pythium ultimum*, or *Phytophthora cinnamomi*, wherein the taxol resistant strain of a natively taxol sensitive fungus is generated by transforming said fungus with a vector encoding a nontaxol-binding beta-tubulin wherein the amino acid at position 219 as numbered in SEQ ID NO:2 is not threonine, or wherein the taxol sensitive strain of a natively taxol-resistant fungus is generated by transforming said fungus with a vector encoding a taxol-binding beta-tubulin wherein the amino acid at position 219 as numbered in SEQ ID NO:2 is threonine;

contacting said composition of matter with the mycelia from said isogenic fungal strains in the presence of labeled taxol; and

determining the degree of competitive inhibition of binding between the beta-tubulins in said isogenic fungal strains and said labeled taxol by said composition of matter;

whereby said composition of matter is determined to possess taxol or taxol-like compounds if it is able to block labeled taxol binding to the taxol-sensitive beta-tubulins in said isogenic fungal strains.

76. A method of developing a taxol-sensitive fungal cell from a taxol-resistant fungal cell comprising

transforming said taxol-resistant fungal cell by introducing a DNA segment encoding taxol-binding beta-tubulin comprising threonine at amino acid 219 as numbered in SEQ ID NO:2;

wherein said transformed fungal cell expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

77. A transgenic taxol-sensitive fungal cell produced from a taxol-resistant fungal cell according to a method comprising

transforming said taxol-resistant fungal cell by introducing a DNA segment encoding a taxol-binding beta-tubulin of *Pythium ultimum* comprising a codon for threonine at amino acid 219 as numbered in SEQ ID NO:4;

wherein said transformed fungal cell expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

78. A transgenic taxol-sensitive fungal cell produced from a taxol-resistant fungal cell according to a method comprising

transforming said taxol-resistant fungal cell by introducing a DNA segment encoding a taxol-binding beta-tubulin of *Phytophthora cinnamomi* comprising a codon for threonine at amino acid 219 as numbered in SEQ ID NO:6 and comprising at least two codons selected from the group consisting of a codon for valine at amino acid 24, a codon for aspartic acid at amino acid 249, a codon for argininc at amino acid 251, a codon for lysine at amino acid 252, a codon for leucine at amino acid 253, a codon for lysine at amino acid 359, and a codon for alanine at amino acid 428 as numbered in SEQ ID NO:5;

wherein said transformed fungal cell expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

79. A method of developing a taxol-resistant fungal cell from a taxol-sensitive fungal cell comprising

transforming said taxol-sensitive fungal cell by introducing a DNA segment encoding a non-taxol-binding beta-tubulin wherein amino acid 219 as numbered in SEQ ID NO:2 is not threonine;

wherein said transformed fungal cell expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

80. A transgenic taxol-resistant fungal cell produced from a taxol-sensitive fungal cell according to a method comprising

transforming said taxol-sensitive fungal cell by introducing a DNA segment encoding a non-taxol-binding beta-tubulin, wherein said DNA segment does not encode threonine at amino acid 219 as numbered in SEQ ID NO:2;

wherein said transformed fungal cell expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

81. A method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising

providing isogenic taxol-resistant fungal cells and taxol-sensitive fungal cells, wherein the difference between the taxol-resistant fungal cells and the taxol-sensitive fungal cells is the identity of amino acid 219 as numbered in SEQ ID NO:2;

contacting said composition of matter with said fungal cells separately;

determining the growth of both strains of fungal cells;

whereby said composition of matter is determined to possess taxol or taxol-like compounds if it is able to inhibit the growth of taxol-sensitive fungal cells but not able to inhibit the growth of taxol-resistant fungal cells.

82. A method for controlling the growth of plant pathogens on a plant comprising

determining if said plant pathogen is taxol-sensitive, wherein said plant pathogen is designated as taxol-sensitive if amino acid 219 as numbered in SEQ ID NO:2 is threonine; and

treating said plant and soil surrounding said plant with a taxol-producing *Pestalotiopsis microspora* if said plant pathogen is taxol-sensitive.